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USE OF HCC-1 AND GLYCOSYLATED HCC-1 TO IMPROVE STEM CELL TRANSPLANTATION

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Abstract:

The patent discloses the human chemokine HCC-1, N-terminally truncated HCC-1 molecules and glycosylated HCC-1 which improve the homing of stem cells into the bone marrow during stem cell transplantation. The patent also provides a procedure for producing the polypeptides by recombinant techniques or chemical synthesis and for producing antibodies against such polypeptide. The patent also discloses the modification of the polypeptide by coupling of amino acid residues and/or chemical groups or deleting amino-acids generating potent derivatives of the polypeptide. Another aspect of the invention provides a combination of the polypeptide of the present invention and a suitable pharmaceutical carrier for providing a therapeutically effective amount of the polypeptide for the treatment of various associated diseases. The patent discloses the use of the HCC-1 molecules to increase engraftment of stem cells in the course of the stem cell transplantation performed in stem cell transplantation related diseases.

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FIELD OF THE INVENTION

The present invention relates to methods of using the human chemokine HCC-1, N-terminally truncated HCC-1 and glycosylated HCC-1 to improve stem cell homing into the bone marrow during stem cell transplantation.

BACKROUND OF THE INVENTION

Hematopoietic stem cells are rare primitive blood cell progenitors that have the capacity to self-replicate, to maintain a continuous source of regenerative cells, and to differentiate, to give rise to various morphologically recognizable precursors of blood cell lineages. These precursors are immature blood cells that cannot self-replicate and must differentiate into mature blood cells. Within the bone marrow microenvironment, the stem cells self-proliferate and actively maintain continuous production of all mature blood cell lineages throughout life.

Bone marrow transplantation is being increasingly used in humans as an effective therapy for an increasing number of diseases, including malignancies such as leukemias, lymphoma, myeloma and selected solid tumors as well as nonmalignant conditions such as aplastic anemias, immunological deficiencies and inborn errors of metabolism. The objective of BM transplantation is to provide the host with a healthy stem cell population that will differentiate into mature blood cells that replace deficient or pathologic cell lineages.

The source of the BM for transplantation may be autologous, syngeneic or allogeneic. Preferred are autologous BM or BM from HLA-matched siblings, but also BM from HLA-nonmatched donors is being used for transplantation.

Complicating factors in BM transplantation include graft rejection and graft-vs-host disease. Since donor T lymphocytes were found to cause GVHD in animals, one of the procedures to prevent or alleviate GVHD consists in removing T cells from the donor BM before transplantation. This can be done by different techniques. Extensive use of T-cell depleted BM effectively prevented GVHD but, unfortunately, resulted in a high rate of graft rejection (10-15 % in HLA-matched recipients and 50 % in HLA-nonmatched recipients) or graft failure (as high as 50 %).

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Another problem in BM transplantation is the difficulty of achieving long-term successful engraftment also when no graft rejection or GVHD occurs. Nowadays, patients which were successfully transplanted have very low levels of stem cells and immature progenitors which generate mature blood cells, compared with healthy individuals.

Stem cells are functionally defined by their ability to home to the bone marrow and to durably repopulate transplanted recipients with both myeloid and lymphoid cells. The processes that mediate homing and engraftment of human stem cells to the bone marrow involve a complex interplay between cytokines, chemokines and adhesion molecules.

Much of our knowledge of the regulation and the hierarchical organization of the hematopoietic system derives from studies in the mouse wherein stem cells are identified and quantified in long-term reconstitution assays. In contrast, our knowledge of the biology of human hematopoiesis is limited, since it is mostly based on in characterize and quantify repopulating stem cells.

Intensive research is being carried out in order to understand the processes that mediate homing and engraftment of human stem cells to the bone marrow. Recently, several groups have established in vivo models for engraftment human stem cells, e.g. into immune deficient mice such as irradiated beige, nude, Xid (X-linked immune deficiency), SCID and non-obese diabetic SCID (NOD/SCID) mice, and in utero transplantation into sheep fetuses which resulted in successful multilineage engraftment of both myeloid and lymphoid cells.

Previously inventors have developed a functional in vivo assay primitive human SCID repopulating cells (SRCs) based on their ability to durably repopulate the bone marrow of intravenously transplanted SCID or NOD/SCID mice with high levels of both myeloid and lymphoid cells ([1, 2]). Kinetic experiments demonstrated that only a small fraction of the transplanted cells engrafted and that these cells repopulated the murine bone marrow by extensive proliferation and differentiation. Furthermore, the primitive human cells also retained the capacity to engraft secondary murine recipients [3]. Transplantation of populations enriched for CD34 and CD38cell surface antigen expression, revealed that the phenotype of SRC is CD34+CD38- [2]. Other repopulating cells may exist since recent studies suggest that immature human CD34- cells and more differentiated CD34+CD38+ cells have some limited engraftment potential [4, 5].

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Accumulating evidence indicates that stem cell homing to the bone marrow is a multistep process. The mechanisms involved in hematopoietic stem cell trafficking have been largely unknown for a long time.

During the past few years, the role of particular secreted (eg, cytokines) and cell-bound proteins (eg, adhesion molecules) in progenitor mobilization and homing has been recognized.[6-9] More recently, it has been shown that cytokines may play a central role in progenitor cell trafficking, particularly in stem cell homing to the bone marrow (BM).[9-12]. Interestingly, extravasation of mature leukocytes during inflammation and homing of immature progenitor and stem cells to the BM may at least partially depend on similar mechanisms [8]. Inflamed tissues and the hematopoietic microenvironment share similarities, such as expression of particular adhesion molecules (E-selectin, vascular cell adhesion molecule-1) on microvascular endothelium [13, 14]. Of particular interest for bone marrow engraftment are the chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4. Treatment of human progenitor cells with antibodies to CXCR4 prevented engraftment into human severe combined immunodeficient (NOD/SCID) mice. *In vitro* CXCR4-dependent migration to SDF-1 of CD34+CD38-/low cells was found to correlate with *in vivo* engraftment and stem cell function [10]. Activation of CD34(+) cells with SDF-1α leads to firm adhesion and transendothelial migration, which is dependent on LFA-1/ICAM-1 (intracellular adhesion molecule-1) and VLA-4/VCAM-1 (vascular adhesion molecule-1). Furthermore, SDF-1-induced polarization and extravasation of CD34(+)/CXCR4(+) cells through the extracellular matrix underlining the endothelium is dependent on both VLA-4 and VLA-5[15].

In view of expanded approach to treatment of many severe diseases by hematopoietic stem cell transplantation, it is highly desirable to understand better the mechanism behind stem cell homing to the bone marrow and repopulation of transplanted hosts in order to obtain stem cells with higher rates of successful and long-term engraftment.

SUMMARY OF THE INVENTION

The present investigation describes a new function the chemokine HCC-1. It has now been found, according to the present invention, that treatment of the murine hematopoietic FDCP-Mix progenitor cells with HCC-1, glycosylated HCC-1 and N-terminally truncated HCC-1 molecules

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induce a chemotactic migration. In this context glycoslylated HCC-1 was identified in a screening for chemotactic activities with subsequent purification of glycosylated HCC-1 from human blood filtrate. Furthermore in an in vivo transplant model using irradiated mice it was found that pretreatment of mononuclear cells containing murine stem cells with HCC-1 improves stem cell engraftment in the bone marrow.

The present investigation thus relates to a method increasing the engraftment of hematopoietic stem and progenitor cells for use in clinical transplantation. The method is related to a pretreatment of transplantable hematopoietic progenitor- and stem cells with HCC-1 prior to transplantation and/or to *in vivo* application of HCC-1 to patients prior-, during, and/or subsequently to stem cell transplantation.

A further aspect of the invention relates to a method for transplantation of immature hematopoietic cells in patients. The patients need conditioning under sublethal, lethal or supralethal conditions, for example by total body irradiation (TBI) and/or by treatment with myeloablative and immunosupressive agents according to standard protocols. For example, a sublethal dose of irradiation is within the range of 3 – 7 Gy TBI, a lethal dose is within the range of 7 – 9.5 Gy TBI, and a supralethal dose is within the range of 9-16.5 Gy TBI. Examples of myeloablative agents are busulphan, dimethyl mileran and thiotepa, and of immunosupressive agents are prednisolone, methyl prednisolone, azathioprine, cyclophosphamide, cyclophosphamide, etc.

The method of the invention is suitable for the treatment of diseases curable by bone marrow transplantation such as malignant diseases, including leukemias, solid tumors, congenital or genetically-determined hematopoietic abnormalities, like severe combined immunodeficiency syndromes (SCID) including adenosine deaminase (ADA) deficiency, osteopetrosis, aplastic anemia, Gaucher´s disease, thalassemia.

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EXAMPLE 1

IDENTIFICATION OF GLYCOSYLATED HCC-1 AS A STEM CELL MIGRATING ACTIVITY.

900 L of human hemofiltrate (HF) for large scale recovery of plasma peptides were obtained from chemotherapy-treated patients with renal failure. Ultrafilters used for hemofiltration had a specified molecular mass cut-off of 20 kD. The sterile filtrate was immediately cooled to 4 °C and acidified to pH 3 to prevent bacterial growth and proteolysis. For peptide extraction the HF was ultrafiltrated a second time. The filtrate was conditioned to pH 2.7 and applied onto the strong cation exchanger, Fractogel TSK SP 650(M), 100 x 250 mm (Merck, Darmstadt, Germany) using an Autopilot chromatography system (PerSeptive Biosystems, Wiesbaden, Germany). Bound peptides were eluted using seven buffers with increasing pH resulting in seven pH-pools. The seven buffers were composed as follows: I: 0.1 M citric acid monohydrate, pH 3.6; II: 0.1 M acetic acid + 0.1 M sodium acetate, pH 4.5; III: 0.1 M malic acid, pH 5.0; IV: 0.1 M succinic acid, pH 5.6; V: 0.1 M sodium dihydrogen phosphate, pH 6.6; VI: 0.1 M disodiumhydrogen phosphate, pH 7.4; VII: 0.1 M ammonium carbonate, pH 9.0. The seven pools (pH pools) were collected and each of them was loaded onto a RP column, 125 mm x 100 mm i.d., Source RPC, 15 μm (Pharmacia) and eluted in a gradient from 100% A (0.01 M HCl in water) to 60%B (0.01 M HCl in 80% acetonitrile). Fractions of 200 mL were collected. In the screening for chemotactic activities using the FDCP-Mix stem cell line the predominant activity was identified in pH pool VI. This chemotactic activity was purified in four further chromatographic steps A to D. (A) Reverse phase chromatography, using a Bakerbond cartrige (47 mm i.d. x 300 mm) with an acetonitril gradient. (B) Size exclusion chromatography using SEC Superdex 16/60 High Load column with the eluent PBS, pH 7.4. (C) Reverse phase chromatography, using a YMC C18 (10 mm i.d. x 250 mm) with an acetonitril gradient. (D)Reverse phase chromatography, using a YMC C18 (4 mm i.d. x 250 mm) with an acetonitril gradient.

Amino acid sequencing by Edman degradation of the purified material revealed the sequence of HCC-1. Mass spectrometric analysis of the isolated material revealed a glycosylated molecule. The isolated molecules revealed molecular weights (M_w) of 9038.15 and 9331.9. Whereas HCC-

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1 (1-74) carries M_w of 8673.09 the increase of the M_w in the isolated molecules was identified as an O-glycosylation of the amino acid Serine in position 7 with N-acetylgalactosamine galactose and with oligosacharide composed of N-acetylgalactosamine galactose and N-acetylneuraminic acid.

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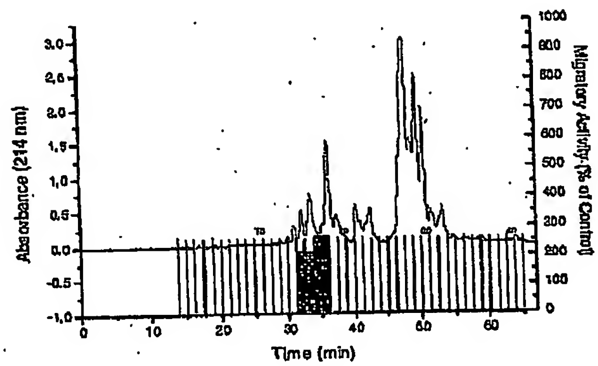
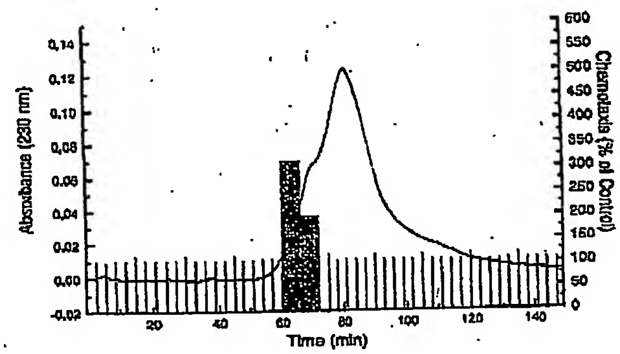
Figure 1



Purification step A: Reverse phase chromatography, using a Bakerbond cartrige (47 mm i.d. x 300 mm) with an acetonitril gradient.

Fig. 2.



Purification step B: Size exclusion chromatography using SEC Superdex 16/60 High Load column with the eluent PBS, pH 7.4.

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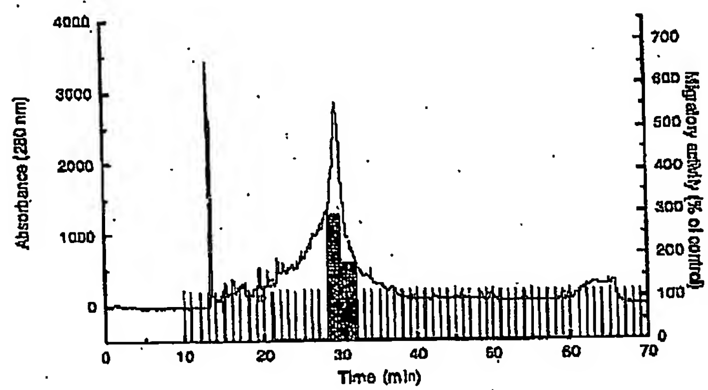
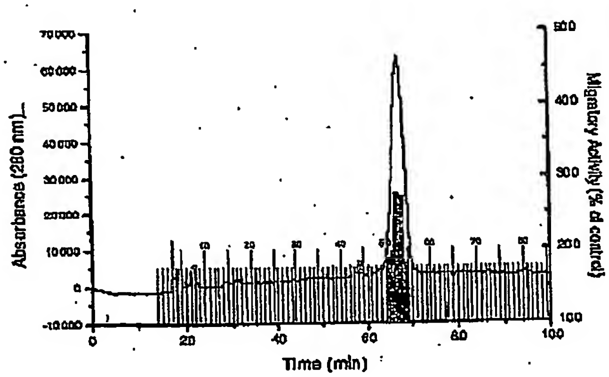
Fig. 3.



Purification step C: Reverse phase chromatography, using a YMC C18 (10 mm i.d. x 250 mm) with an acetonitril gradient.

Fig. 4.



Purification step D: Reverse phase chromatography, using a YMC C18 (4 mm i.d. x 250 mm) with an acetonitril gradient.

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EXAMPLE 2

CHEMOTACTIC ACTIVITY OF HCC-1 MOLECULES TO THE MURINE FDCP-MIX STEM CELL LINE

Fig 5: Chemotactic activity of HCC-1 (1-74) and glycosylated HCC-1 (1-74) on FDCP-Mix cells

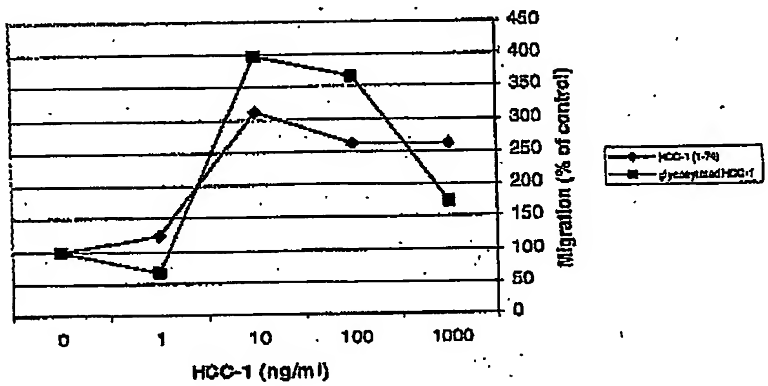


Fig 6: Chemotactic activity of HCC-1 (1-74) and HCC-1 (9-74) on FDCP-Mix cells

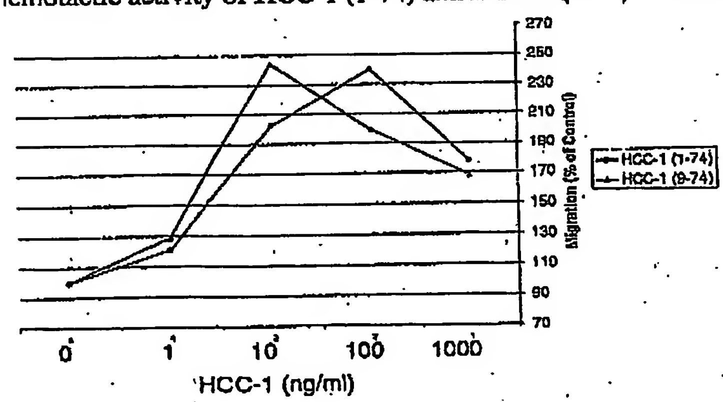


Figure 5 and 6: FDCP-Mix cells were subjected to in vitro chemotactic assays. Chemotaxis was assessed in 96-transwell chambers (Neuroprobe, Cabin John, MD) by using

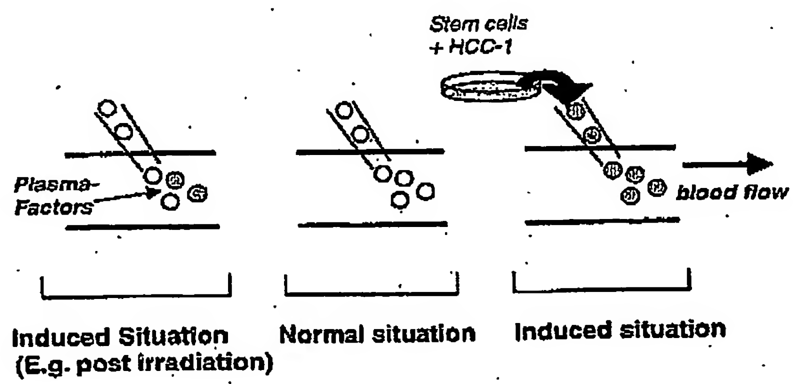
polyvinylpyrrolidone-free polycarbonate membranes (Nucleopore, Neuroprobe) with 5-μm pores. Four hundred microliters of IMDM medium was added to the bottom of the well, and was supplemented with varying concentrations of HCC-1 molecules. 200 μl of IMDM medium containing 100.000 FDCP-Mix cells were added to the upper wells of the chemotaxis chamber. All assays were carried out in triplicate, and the migrated cells were counted in 4 randomly selected fields at 63-fold magnification after migration for 14 h.

EXAMPLE 3

MODULATION OF HOMING MECHANISMS BY PREINCUBATION WITH HCC-1 *IN VITRO*

Enriched Mononulcear cells, CD34+ progenitor cells from human cord blood, mobilized peripheral blood, or bone marrow are incubated with HCC-1 in concentrations between 100 pM and 10 μM for a time period which is between 5 minutes and 12 hours.

Fig. 7: Concept of the modulation of homing mechanisms by preincubation with HCC-1.



After preincubation stem cells are transplanted into the blood flow. In a competitive repopulation model using Ly 5.1 and Ly 5.2 mice it was shown that preincubation of the cells gives an advantage in the engraftment of the bone marrow over cells which were not treated with HCC-1.

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CLAIMS

1. Chemokine glycosylated HCC-1

HCC-1(1-74)

```
          10         20         30         40         50         60         70
           |          |          |          |          |          |          |
TKTESSSRG PYHPSECCFT YTTYKIPRQR IMDYYETNSQ CSKPGIVFIT KRGHSVCTNP SDKWVQDYIK DMKEN
      |
      R
```

whereby R is an oligosaccharide composed out of N-acetylgalactosamine galactose or an oligosacharide composed out of N-acetylgalactosamine galactose and N-acetylneuraminic acids.

and its biologically active fragments, analogs and derivatives, in particular amidated, acylated, and/or phosphorylized derivatives

wherein the two cystein residues in positions 16 and 40 linked together by a disulfide bond and wherein the two cystein residues in positions 17 and 56 are linked together by a disulfide bond.

2. A polypeptide having at least 90% identity to the polypeptide sequence of claim 1.

3. The processed chemokine according to claims 1 and claim 2 (a) wherein the N-terminus is modified by coupling a chemical group generating a chemokine having the structure of [Glyoxyloyl1]PHC 1-Pentane oxime, Nonanyl-PHC, [Glyoxyloyl1]PHC 1-Heptane oxime, [Glyoxyloyl1]PHC 1-Hexane oxime, [Glyoxyloyl1]PHC 1-Pentene oxime or Nonaoyl-PHC and wherein the modification is influencing the biological activity of PHC or (b) wherein amino acid residues of the N-terminus or of the C-terminus are deleted.

4. The chemokines according to claims 1, 2, and 3 wherein one or more lysine, histidine, glutamate, aspartate, or cysteine residues of the chemokine are modified by coupling a

chemical group having the structure of polyethylenglycol and wherein this modification is increasing the plasma half-life time of HCC-1.

5. An antibody against an amino acid sequence of claims 1, 2, 3, or 4.

6. A diagnostic agent containing polyclonal or monoclonal antibodies against chemokine HCC-1 of claims 1 to 4.

7. A drug containing chemokine HCC-1 of claims 1, 2, 3, 4 or the antibody of claim 5.

8. A process for producing a polypeptide comprising polypeptides named in claim 1 to 4 using recombinant techniques or chemical synthesis.

9. A process for producing cells capable of expressing a polypeptide comprising in claims 1, 2, 3, 4.

10) Use of HCC-1 or molecules claimed in claims 1,2,3, and 4, HCC-1 molecules without glycosylation and N-terminally truncated HCC-1 molecules, especially HCC-1 (2-74), HCC-1 (3-74), HCC-1 (4-74), HCC-1 (5-74), HCC-1 (6-74), HCC-1 (7-74), HCC-1 (8-74), HCC-1 (9-74), HCC-1 (10-74), HCC-1 (11-74) and HCC-1 (12-74) to increase engraftment of stem cells.

11) Use of HCC-1 molecules according to 10) for transplantation of progenitor and stem cells.

12) Use of HCC-1 molecules according to 10) for treatment of progenitor- and stem cells prior to transplantation.

13) Use of HCC-1 molecules according to 10) for *in vivo* application of such a molecule into patients which are receiving stem cell transplantation prior to and/or in the course of stem cell transplantation.

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14) Use of HCC-1 molecules according to claims 10) to 13) wherein the host patient are not conditioned.

15) Use of HCC-1 molecules according to claims 10) to 13) wherein the host patient is conditioned under sublethal, lethal, or supralethal conditions.

16) Use of HCC-1 molecules according to claim 15) wherein sublethal, lethal, or supralethal conditions include treatment with total body irradiation, optionally followed by treatment with myeloablative or immunosuppressive agents.

17) Use of HCC-1 according to claim 15) wherein sublethal, lethal, or supralethal conditions include myeloablative or immunosuppressive treatment without total body irradiation.

18) Use of HCC-1 according to claims 10) to 17) for the transplantation of hematopoietic progenitor and stem cells, umbilical cord blood and placental stem and progenitor cells, liver stem and progenitor cells (oval cells), mesenchymal stem and progenitor cells, endothelial progenitor cells, skeletal muscle stem and progenitor cells (satellite cells), smooth muscle stem and progenitor cells, intestinal stem and progenitor cells, embryonic stem cells, and genetically modified embryonic stem cells, adult islet/beta stem- and progenitor cell, epidermal progenitor and stem cells, keratinocyte stem cells of cornea, skin and hair follicles, olfactory (bulb) stem and progenitor cells and side population cells from diverse adult tissues.

19) Use of HCC-1 molecules according to claims 10) to 18) for the treatment of leukemias, lymphoproliferative disorders, aplastic anemia, congenital disorders of the bone marrow, solid tumors, autoimmune disorders, inflammatory diseases, primary immunodeficiencies, primary systemic amyloidosis, systemic sclerosis, heart diseases, liver diseases, neurodegenerative diseases, multiple sclerosis, M. Parkinson, stroke, spinal cord injury diabetes mellitus, bone diseases, skin diseases, replacement therapy of the skin, retina or cornea, other congenital disorders, vessel diseases like atherosclerosis or cardiovascular disease.

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